Attorney Work Document

Internal Electrophysiological Studies

The first full-scale study addressing the CNS effects of cigarette smoking was completed in 1979. The title of the study was: The Effects of Cigarette Smoking on the Early, Late and after-Discharge Components of the Visual Evoked Response. The study was undertaken because two earlier reports (Hall et al, 1973; Friedman et al, 1974) suggested that components of the response are modifiable by cigarette smoking.

The visual evoked response (VER) is a cortically recorded evoked potential (EP) elicited by brief flashes of monochromatic light. Like most EPs, the VER is an averaged response obtained from repeated stimulation. The late components of the response have been shown to be modifiable by exogenous agents. In particular, it was demonstrated that following smoking, the amplitudes of the IV-V component (Hall et al, 1973) and the VI-VII component (Friedman, et al, 1974) of the response were increased.

The purpose of our study was to attempt to replicate and extend the two earlier studies on the VER and smoking

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Eleven volunteers served as subjects for the experiment. They were tested after both overnight and 1 h. smoke deprivation. VERs were recorded immediately before and just after smoking one of two experimental cigarettes. The cigarettes differed in nicotine delivery (0.14 and 1.34 mg/cigt) but were relatively invariant in other smoke constituents. a sham smoking control was also included in the study. The cigarettes were smoked on separate days with order of smoking randomized for each subject. In addition to VERs, we also recorded heart rate (pulse) and expired Carbon Monoxide (CO).

We found that under the 1 h smoke deprivation condition, smoking the 1.34 mg cigarette resulted in an amplitude increase in three of the four late components of the VER, and that that there was a trend of an amplitude increase in fourth component. Smoking a 0.14 mg cigarette had no effect on the VERs. Smoking either cigarette had no effect on the VER when the subjects were overnight smoke deprived.

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These results complement those of the Hall and the Friedman groups in that we found amplitude increases in both the IV-V and the VI-VII. However, our results are more robust in that all four of the late components of the VER were enhanced.

In the study just described, we were surprised to find that the amplitudes of the late VER components were increased following smoking when the subjects were 1h smoke deprived but not when subjects were over night smoke deprived. We expected that whatever effects we obtained would be magnified the longer the deprivation interval. This issue was addressed in the VER II study. We reasoned that if VER amplitudes were not increased following the smoking of single cigarette when the subjects were over night smoke deprived, perhaps the response would be increased when the subjects smoked several cigarettes in succession.

To test this hypothesis, we recorded VERs in 7 volunteers prior to and immediately after smoking 3 cigarettes (1.34 mg/cigt) in succession. The subject were over night smoke at the beginning of the experiment.

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as a control, the subjects abstain smoked three unfit cigarettes while overnight smoke deprived.

The results of the study failed to support our hypothesis. a significant decrease in the amplitudes of most VER components was obtained both after smoking and in the control condition. These data indicate that repeated testing, in and of itself depresses VER amplitudes. The data further indicate that cigarette smoking under overnight deprivation conditions do not reverse this depressant effect.

a study entitled "Long-Term Smoke Deprivation and the Electrical activity of the Brain" sought to determine what, if any, changes would occur in the VER when people stopped smoking. Previous studies using smoke deprivations of 12 and 36 h had suggested that VER amplitudes are depressed relative to smoking baselines (Kaul et. al, 1973; Friedman et al 1974). We (1) wanted to replicate these findings and (2) extended the deprivation period for days or weeks by using people who, for various reasons were giving up smoking. We also tested a group of non-smoking

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control subjects in order to assess the stability of the VER over time, relative to quitters.

Our hypotheses were that if the cessation of cigarette smoking resulted in a permanent reduction in VER amplitudes, this would indirectly imply a return to some kind of pre-smoking baseline. That is, the responses were depressed before the person ever began to smoke. If, on the other hand, the VER remained depressed for some period of time and then gradually recovered to pre-quitting levels, this would indirectly suggest a withdrawal phenomenon of some time.

Finally we were interested in some of the behavioral phenomena associated with smoking cessation. In particular we were interested in changes in appetite, sleep and mood.

The subjects used in this experiment were 12 volunteers, seven of whom were smokers who, for one reason or another expressed a desire to quite (quitters), and five of whom were nonsmokers. Prequitting measures (VER, mood scale, etc) were taken on the day on the day before the quitters stopped smoking. We then repeated the experiments, one, two and

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three days postquitting. Next, we repeated the experiment one week postquitting. Thereafter, most subjects were tested at monthly intervals. an identical schedule was followed for the nonsmokers.

There were no dramatic changes in the VER postquitting. One day post quitting we found a transient (one day) increase in the latency of one VER component in the quitters. This, however, could be statistical artifact due to the large number of tests that were performed. Amplitudes remained remarkably stable post quitting, contradicting the finding of other laboratories (Ball et al, 1973; Friedmeen et al, 1984).

The mood scale indicated that there were no mood changes after quitting for the first several days post-quitting, the quitters reported feeling more tired and irritable. By one week post quitting these symptoms had largely disappeared. The most notable change post quitting were appetite increases, where the quitters reported cravings for high calorie foods such as sweets and potatoe chips.

In summary quitting resulted in

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no remarkable changes in the V.E.R. The only significant changes were in sleep patterns and behaviors.

Taken as a group, these studies indicate that under certain conditions, changes in the VER can be detected after smoking (eg, amplitude increases). However, the VER suffers from high inter and intra subject variability. This means that it is often difficult to replicate previous finds For this and other reasons, we abandoned VER studies and undertook studies on auditory evoked potentials (AEPs).

The AEP is cortically recorded evoked potential elicited by brief presentations of pure tone pips delivered through head sets. Like most other EPs the AEP is an averaged reponse obtained by repeated stimulation. The AEP waveform is considerably easier to measure in that it has far fewer components than the VER. Hence, we believed it would be more reproducible and, therefore, more reliably analysed.

At the time the study was conducted, we only ~~knew~~ of two experiments on the effects of nicotine or smoking on the AEP. In an animal experiment (Guha & Pradhan, 1976) it was found

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that low systemic doses of nicotine increased AEP amplitudes while higher doses and intra-cerebral administration depressed amplitudes. In the only human study of that time, it was found that cigarette smoking tended depress AEP amplitudes (Friedman, et al, 1974). Since VERs are enhanced, while AEPs are depressed by smoking we thought that smoking might be exerting a selective rather than generalized effect on the brain. We were also interested in whether any effects on the AEP were influenced by smoke deprivation. Finally, we wanted to compare the response in smokers and nonsmokers.

Ten smokers and 10 nonsmokers were tested in the study. The smokers were tested when they were not smoke deprived and again when they were over night smoke deprived. The test cigarette delivered 1.34 mg/cgt nicotine.

In contrast to the findings of Friedman et al (1974), we found that cigarette smoking had little or no effect on AEP amplitude AEP amplitudes were depressed post smoking relative to pre-smoking values, but

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this occurred both when a cigarette was really smoked and when a cigarette was sham smoked. These results indicate that repeated testing and not smoking results in decreases in AEP amplitudes

Smoke deprivation had no effect on any AEP measures. Also, the AEP did not differ smokers and nonsmokers. Taken as a whole, these findings suggest that cigarette smoking has no effects on the AEP

We studied the effects of cigarette smoking on second type of AEP called the brainstem auditory evoked potential (BAEP). Like the AEP, the BAEP is recorded from the scalp following acoustic stimulation. In contrast to the AEP, the neural generators for the BAEP are in the brainstem and not the cortex. Thus, cortical (or scalp) recordings represent effects volume conducted from their source of origin.

The BAEP consists of seven components or peaks. A lot more is known about the neural origins of these components than about the origins of the AEP for example, the first component of the BAEP is known to reflect neural activity in the auditory nerve, the

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second component reflects the neural activity of nuclei in the medulla, etc (Stockard et al, 1978).

It has been shown that peripheral administration of nicotine in rats produces a decrease in the amplitude of components of the BAEP. Since much is known about the neural origins of components of the BAEP, this led us to believe that we might be able to determine, in part, where in the brain cigarettes were having an effect. For example, if smoking a cigarette affected the first component we could say that smoking affected activity in the auditory nerve.

Ten smokers served as subjects in the experiment. On different occassions, the subjects were required to smoke a high nicotine delivery cigarette (1.34 mg/cig) a low nicotine delivery cigarette (0.14 mg/cig), or sham smoke an unlit. The deprivation conditions were overnight smoke deprived and not smoke deprived. Thus, each subject was tested six times (ie 3 smoking × 2 deprivation conditions)

The stimuli were for eliciting the BAEPs were clicks delivered through headphones at a rate of 11/sec. BAEPs were recorded before and just after

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smoking or sham smoking.

We found that cigarette smoking had no effect on the amplitudes of any components of the BAEP. We observed a statistically significant latency increase in one component (peak IV). However, this increase was obtained for the low nicotine high nicotine and sham smoking conditions, indicating that smoking was not responsible for the latency increase. From these data we concluded that the BAEP is not modified by cigarette smoking.

Up to this point (early 1981) we still had no reliable measure to assess the effects of cigarette smoking on the CNS. This changed dramatically when we came across an EP called the pattern reversal evoked potential (PREP). The PREP is a type of VER which uses a pattern displayed on a video monitor as a stimulus. The pattern in this case, is a black and white checkerboard as the name implies, the positions of the black and white checks reverse. The rate of reversal is usually about two per second. The waveform of interest is a averaged response obtained from 100-200 reversals.

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The beauty of the PREP is that it is remarkably stable and reproducible both within and between individuals. For this reason it has become a standard tool of neurologists for assessing the health of the CNS. In particular, it has proven to be highly effective in assessing demyelinating diseases such as multiple sclerosis (Milner, et al 1974)

When we began our work on the PREP in mid 1980, little was known about the effects of exogenous [illegible] on the PREP. Therefore, we had no idea how modifiable the PREP was by such agents. In particular, there had been no studies on the effects of cigarette smoking on the PREP.

Our first study, then, was to determine whether the PREP would be affected in some manner by cigarette smoking. The question asked was simply: "What would the pattern of the PREP look like relative to pre-smoking baselines.

Ten volunteers who were regular smokers participated in the study. At the time of testing they were overnight smokers deprived. The cigarettes that were used varied in nicotine delivery, and

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1.34 mg/cigt) but were relatively invariant in the delivery of other smoke constituents. On separate occasions the subjects were required to smoke the 0.14 mg cigarette, the 1.34 mg cigarette, or to sham smoke an unlit cigarette. Baseline PRE Ps were recorded just prior to smoking and post smoking PRE Ps were recorded immediately after smoking. A controlled smoking procedure was used where the experimenter specified how to puff, when to puff and how many puffs to take.*

Three components of the PRE Ps waveform were measured: N1, P1 and N2. N and P refer to polarity being negative and positive. The subscripts refer to the ordinal position of the component with N1, for example, occurring before N2. Two parameters, latency and amplitude, of each component were evaluated. Latency is a time parameter and refers to how soon after stimulus presentation (in this case, a puff) a component occurs. Amplitude is a magnitude parameter indicating how large a component was. In this experiment the amplitude of a component was measured relative to a pre-stimulus baseline.

* This was done to ensure that the

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An analysis of the data revealed that following the smoking of the 1.34 mg cigarette there was a statistically significant decrease in the amplitude of the N_1 component. No statistically significant effects were obtained on N_1 following the smoking the 0.14 mg cigarette or following sham smoking.

Statistically significant decreases in the latency of the P_1 component were obtained following the smoking of the 1.34 mg cigarette. Smoking the 0.14 mg cigarette and sham smoking had no statistically significant effect on P_1 latency. Smoking had no effect on other latency and amplitude values.

One other finding is of note. At the time we conducted this study we thought we observed an additional component in the baseline (i.e. smoke deprived) PREP. This component appeared to be a peak between P_1 and N_2. This component seemed to disappear after smoking. We had no idea at the time what this component represented. Only after nearly a dozen years of additional experience recording

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the response, two possibilities emerge. The first possibility, given its latency (~ 125 msec) is that it is a depressed N2 which recovers after smoking. The second possibility has to do with smoke deprivation induced slow waves ... the amount of averaging is insufficient slow waves may ... PREP ... additional component. Naturally, ... smoking the slow waves ... and the additional component ... seemingly disappear.

The data, taken as whole, indicate the PREP can be used ... effects of cigarette smoking ... CNS. Furthermore, the effects ... appeared to be due to nicotine, ... only the 1.34 mg nicotine ... produced statistically significant differences.

A question that immediately arose from the previous study, ... mild CNS stimulants other than nicotine also have ... effect on the PREP? The logical choice for a comparable mild CNS stimulant was caffeine.

Ten volunteers participated in the study. All were non-smokers, but regularly

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consumed caffeine in several different forms. The tests were conducted in the morning following overnight abstinence from all caffeine containing substances (e.g. coffee, tea, cola, chocolate, certain headache preparations, etc.

Baseline PRTPs were first recorded in all subjects. The subjects were then given capsules containing 300 mg caffeine (ie the amount of caffeine in about three cups of strong coffee) or fructose (a sugar derived from fruit). Post ingestion PRTPs were recorded 1 h later.

Caffeine ingestion produced statistically significant decreases in the latency of the N_1 component of the PRTP. Caffeine also produced a statistically significant decrease in the latency of P_1. No statistically significant effects were observed following fructose ingestion.

The effects observed with caffeine were, in some respects, similar to the effects produced by nicotine. For example, P_1 latency decreased in both cases. However, caffeine ingestion also resulted in a decrease in N_1 latency, while nicotine resulted in an increase in N_1 amplitude.

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Our third PREP study was entitled: "Sustained Stimulation and Pattern Reversal Visual Evoked Potentials: Smoking Effects"

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